**2017 International Symposium on Cocoa Research (ISCR)c, Lima, Peru, 13-17 November 2017**

# EFFECTS OF MICROCLIMATIC VARIABLES ON THE ONSET OF SYMPTOMS AND SIGNS OF *Moniliophthora roreri* FOR THREE CACAO CLONES IN A RANGE OF INCOMPLETE RESISTANCE

M.E. Leandro-Muñoz[1], P. Tixier[1,2], W. Phillips-Mora[1], S. Maximova[3], J. Avelino[1,4,5].
[1] Agroforestry and Sustainable Agriculture Department, Division of Research and Development, Tropical Agricultural Research and Higher Education Center, Turrialba, Cartago, Costa Rica
[2] CIRAD, Persyst, UPR 26, TA B-26/PS4, Boulevard de la Lironde, 34398, Montpellier Cedex 5, France
[3] Department of Horticulture, 306 Wartik Lab, Pennsylvania State University, University Park, PA 16802, USA
[4] CIRAD, UPR Bioagresseurs analyse et maîtrise du risque, F-34398 Montpellier, France
[5] IICA-PROMECAFE, Coronado, San José, Costa Rica

Moniliophthora Pod Rot (MPR), caused by the fungus *Moniliophthora roreri* (Cif.) Evans et al is one of the main limiting factors of production in Latin America. Combating MPR is difficult due to the time-consuming and high cost recommended practices. This limitation is due to the current insufficient information on the biology and epidemiology of the pathogen. This research aims to compare MPR development, symptoms onset of the disease and fungal sporulation for three cacao clones in a range of incomplete resistance—Pound-7 (highly susceptible), CC-137 (moderately resistant) and CATIE-R4 (highly resistant)—and understand the influence of different microclimatic variables on this development. A total of 10,054 pods of 5-10 cm length were labelled during 55 weeks. Pods were observed throughout their lifetime: healthy, diseased with no sporulation, diseased with sporulating lesions, harvested. Incidence curves were built for all of the 55 generations of pods observed. Generations with nonconventional clonal behavior were selected in order to illustrate our hypothesis that environment, especially climate, could affect cacao's incomplete resistance to MPR. Differences in resistance among these clones lie in the number of resistant genes accumulated; however, the resistance of the three may be affected under certain environmental condition. Then, using GLMM, GLM and AIC surfaces we determined the specific period (when and for how long) where each microclimatic variable better explained the disease development. These new variables were combined in a complete GLMM and GLM, where only significant variables were retained. Water-related variables and temperature determine the symptoms expression for the susceptible clones, while, for the resistant clone CATIE-R4, only temperature showed up as explicative variable due to low numbers of CATIE-R4 pods showing symptoms. According to our models, there are two important events where resistance strategies could be developed for the cacao resistance strategy: fungal germination and penetration, where PAMP-triggered immunity (PTI) could be activated; and the symptoms onset, where the effector-triggered immunity (ETI) could occur. Success of these two events responds to the effect of humidity and temperature, respectively. We considered that none of the clones presents PTI as a defense mechanism against spore germination and penetration. Host resistance mechanisms resulting from the ETI are triggered internally and against colonization, where temperatures influence the success of these strategies. CATIE-R4 resistance strategy consists of the interruption of fungal colonization as an ETI strategy. This interruption also avoids fungal reproduction since the fungus has difficulties sporulating over CATIE-R4 pods, causing inoculum suppression.

## INTRODUCTION

The plant immune system differs from the mammal somatic adaptive immune system because it lacks mobile defense cells. Instead, the plant immune system depends on every cell's innate immunity and signal transduction cascades triggered from infection sites. There are two branches of this system; the first one is the pattern-recognition receptor proteins (PRRs) inserted in the cell membrane, which recognize and respond to pathogen-associated molecular patterns (PAMPs), activating PAMP triggered immunity (PTI). The second branch acts largely in the cell's interior. There, most resistance genes (R genes), as members of the nucleotide-binding (NB), leucine-rich repeat (LRR) domain of NOD-like receptors (NLR), encode for effector proteins that can activate the effector-triggered immunity (ETI). This last type of immunity is only effective on obligate biotrophs or hemobiotrophs (Jones and Dangl 2006).

Plant genetic resistance to pathogens could be complete or incomplete. Complete resistance is determined by a single or a few host genes. According to Waller *et al.* (2002), this type of resistance may be overcome by genetic changes in the pathogen. Incomplete resistance is mostly determined by multiple genes with quantitative effects that trigger different reactions to protect the plant material from the fungal damage, reducing the severity of the disease without totally excluding it. The selection pressure of this kind of resistance is lower than the one exerted by major genes of complete resistance. However, incomplete resistance can be affected by the environmental conditions, especially climate (Zadoks and Van Leur 1983).

This situation is well-known in the case of the pathosystem *Coffea Arabica—Mycena citricolor.* All arabica coffee cultivars are susceptible to *M. citricolor;* however, different degrees of susceptibility/resistance have been detected. Cultivars derived from the Timor hybrid, which are resistant to coffee rust caused by *Hemileia vastatrix*, apparently are more susceptible to *M. citricolor* than the others, impeding their use even in certain suboptimal environments for the disease. Nonetheless, as soon as environmental conditions, especially humidity, approach optimum for the fungus, the differences in susceptibility lessen (Wang and Avelino 1999; Avelino *et al.* 2007). Similarly, Eskes (1982) reports that coffee resistance to coffee rust also varies according to light intensity in one-year-old coffee seedlings. In other pathosystems, Bonman (1992) has stated that incomplete resistance to rice blast disease is greatly affected by the environment, specifically by night temperatures, duration of leaf-wetness, nitrogen fertilization, soil type and water deficit. Rubiales *et al.* (2012) have also found that faba bean resistance against *Ascochyta fabae* is unstable across environments, the result of a multi-environmental analysis evaluation to find sources of resistance in a germplasm collection. Similarly, different behaviors of host genotypes against pathogens in relation with environment (different sites, years, inoculum pressure) have also been highlighted in the case of the cassava pathosystem—*Xanthomonas axonopodis* pv. *Manihotis* (Banito et al. 2008), or *Euthamia graminifolia—Coleosporium asterum* (Price *et al.* 2004).

Therefore, genotype x environment interactions for incomplete resistance seem to be the rule. The mechanisms involved in the expression of incomplete resistance are not clear. Bonman (1992) has considered that environment may act at different levels, over the host physiology, the pathogen or the interaction of these two components. On that issue, Price *et al.* (2004) have developed an interesting proposal by fitting curves of infections levels as a function of inoculum density to logistic models for different genotypes. These authors stated that different shapes of the fitted curves can highlight different mechanisms of environmental influence on incomplete resistance. According to Parlevliet (1979), incomplete resistance can affect the frequency of host penetration, the rate of development from propagule to lesion and/or the number of propagules produced on these lesions. Each of these stages can be influenced by meteorological factors in a different manner, explaining the genotype x environment interactions observed.

Genotype x environment interactions raise question about the specific resistant cultivars that can be distributed in different environments. The use of specific cultivars exhibiting incomplete resistance can be invalidated in specific environments where this resistance is not efficient. This question is also valid for the case of the pathosystem *Theobroma cacao—Moniliophthora roreri,* for which no complete resistance has been found. However, several degrees of incomplete resistance have been highlighted in different promising host genotypes (Porras Umaña 1985; Phillips 1986) whose resistance could be influenced by different meteorological conditions.

In this study, we analyzed the influence of microclimate on the expression of the incomplete resistance to Moniliophthora pod rot (MPR). We used three different, highly productive clones, with different levels of incomplete resistance characterized by the MPR incidence from 2007 to 2011: 1) Pound-7, with an average incidence of 86%, 2) CC-137, with an average incidence of 43%, and 3) CATIE-R4, with an average incidence of 12%. These clones, on average, maintain their level of resistance, but in specific years, their behavior changes

drastically. These differences sometimes have been observed for the three clones and in other cases, only one of them was affected. From this study, we will deduce whether different mechanisms of incomplete resistance are involved in these host genotypes and the conditions for their deployment in the field. This research aims to compare MPR development, symptoms onset of the disease and fungal sporulation for three cacao clones in a range of incomplete resistance—Pound-7 (highly susceptible), CC-137 (moderately resistant) and CATIE-R4 (highly resistant)—and understand the influence of different microclimatic variables on this development.

## MATERIALS AND METHODS

This research was carried out to complement the study done by Leandro-Muñoz *et al.* (2017), where the Pound-7 clone was evaluated. Complementary data were collected at the same place and in the same moment. However, in this research, we only include the observations from the other two clones: 1) CC-137, a MPR moderately resistant clone (32% of average incidence) with an average production of 990 kg/ha/yr and 2) CATIE-R4, a MPR highly resistant clone (9% of average incidence) with an average production of 1336 kg/ha/yr. All of these clones are considered as highly productive, thus ensuring the presence of pods throughout the year. This information was obtained from a historical data average of 11 years from the CATIE Cacao Improvement Program (Phillips-Mora *et al.* 2013).

We followed the same methodology than Leandro-Muñoz *et al.* (2017), however, some changes were applied to this study, described below.

### Studied periods

The first stage of the analysis considers the identification of the period of major status-change occurrence from healthy pods to diseased pods without sporulation (H→D) and from diseased pods without sporulation to pods with sporulated lesions (D→S). Clones CC-137 and CATIE- R4 had the most number of status changes in the same periods. For H→D, we found the highest number of status changes in a period of 10 days, from 40 to 50 days after tagging (d.a.t) and for D→S, 60 to 70 d.a.t. (Figures 1 and 2). For the CC-137 clone, 14.6% of H→D changes occurred during the 40 to 50 d.a.t. period and 19.7% of D→S changes occurred during the 60 to 70 d.a.t. period (Figure 1). For the CC-137 clone, 14.6% of H→D changes occurred during the 40 to 50 d.a.t. period and 19.7% of D→S changes occurred during the 60 to 70 d.a.t. period (Figure 2).

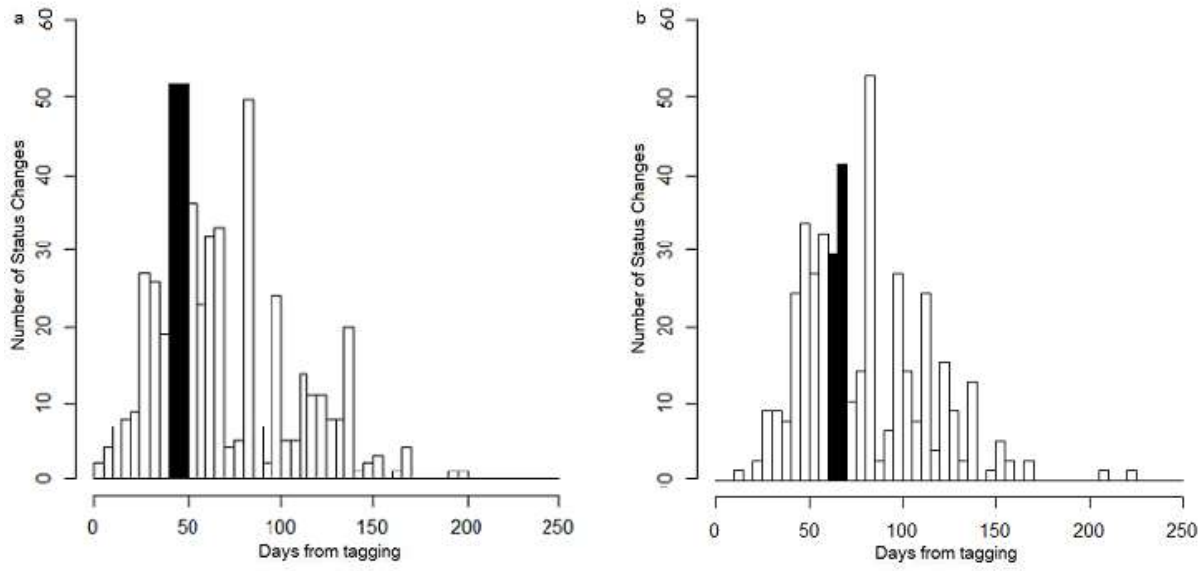



*Figure* 1. *Histograms for the selection of the studied periods for CC-137: a. corresponds to pod status change from healthy to diseased with no signs of sporulation, b. corresponds to pod status change from diseased with no signs of sporulation to sporulated lesions.*

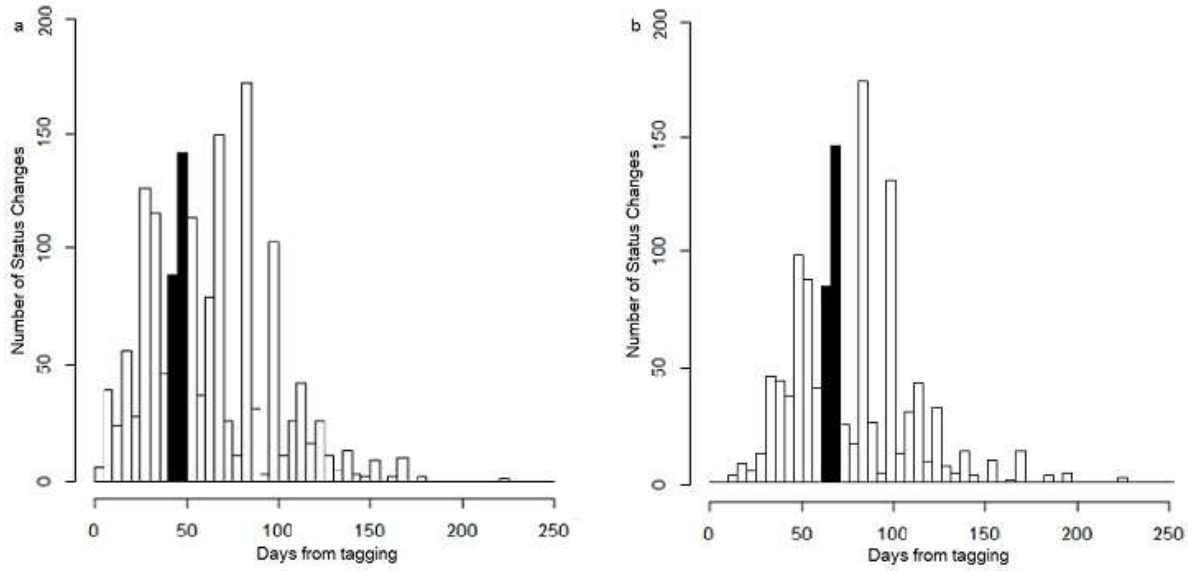



*Figure* 2. *Histograms for the selection of the studied periods for CATIE-R4: a. corresponds to pod status change from healthy to diseased with no signs of sporulation, b. corresponds to pod status change from diseased with no signs of sporulation to sporulated lesions.*

**Statistical analyses**

Descriptive analyses were first conducted to highlight different behaviors of disease incidence of each studied clone. For that purpose, incidence curves were built for all of the 55 generations of pods observed in

this experiment. Generations with nonconventional clonal behavior were selected in order to illustrate our hypothesis that environment could affect cacao's incomplete resistance to MPR.

The methodological approach was almost the same as implemented by (Leandro-Muñoz *et al.* 2017). However, in the statistical analyses, a change was made. For the single predictor analysis and the complete analysis, a generalized linear model (GLM) was used instead of a generalized linear mixed model (GLMM), meaning that *generation* was not included in these analyses as a random factor since models did not converge with the GLMM analyses. This could possibly be due to the fewer number of status changes that occurred for CC-137 and CATIE-R4, which are more resistant than Pound-7.

***Single predictor GLM analysis***

In this part, *mean relative humidity*, *maximum relative humidity* and *amplitude of relative humidity* were not excluded from the analyses. Dates and durations of every variable were selected from Figures 3 and 4 for the CC-137 clone and from Figures 5 and 6 for the CATIE-R4 clone.

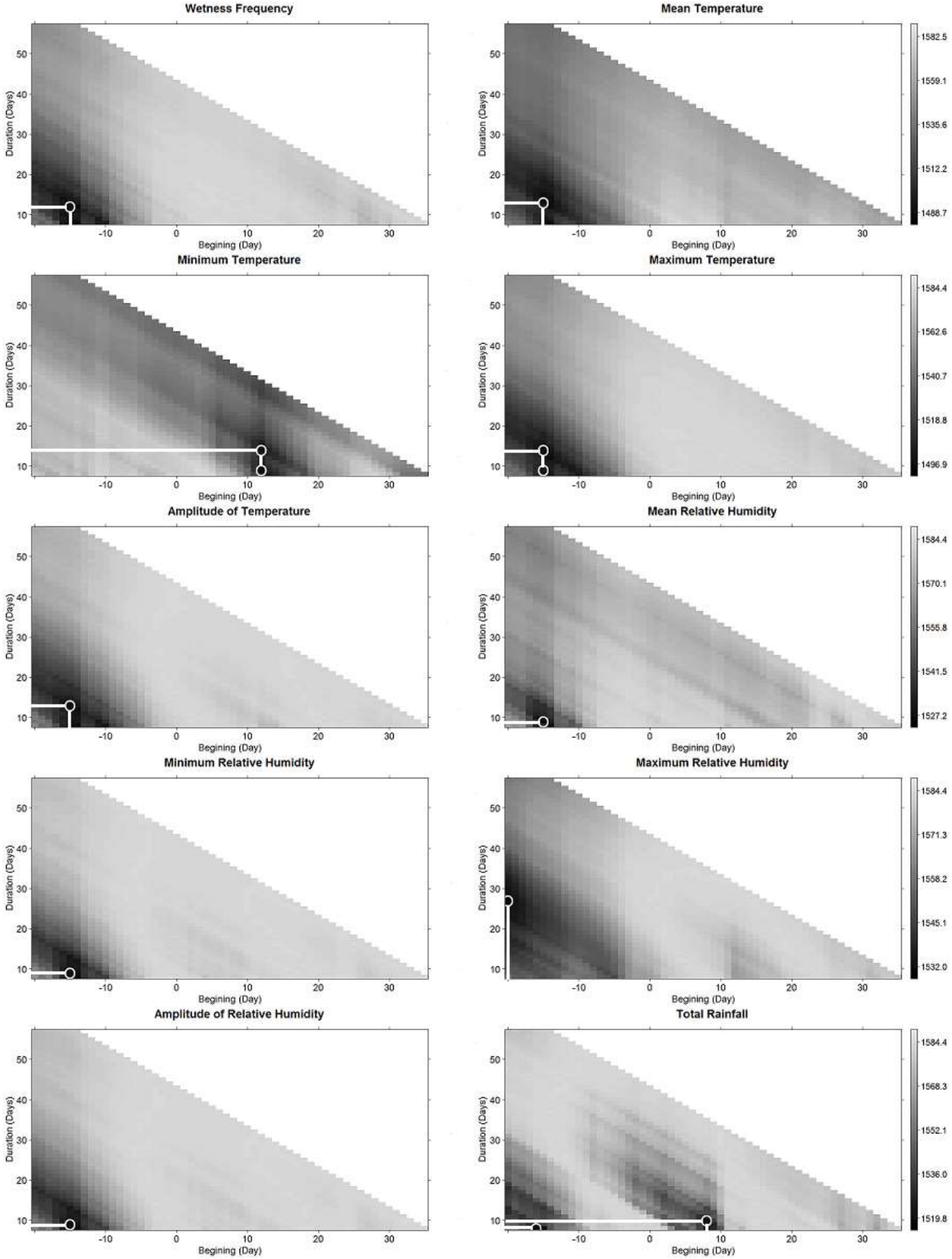

Wetness Frequency
Mean Temperature
Minimum Temperature
Maximum Temperature
Amplitude of Temperature
Mean Relative Humidity
Minimum Relative Humidity
Maximum Relative Humidity
Amplitude of Relative Humidity
Total Rainfall
Duration (Days)
Begining (Day)
10
20
30
40
50
-10
0
10
20
30
1582.5
1559.1
1535.6
1512.2
1488.7
1584.4
1562.6
1540.7
1518.8
1496.9
1584.4
1570.1
1555.8
1541.5
1527.2
1584.4
1571.3
1558.2
1545.1
1532.0
1584.4
1568.3
1552.1
1536.0
1519.8

*Figure* 3. *Period of influence of each daily variable on pod status change, from healthy to diseased with no signs of sporulation, 40 to 50 days after tagging, for CC-137. By period of influence we meant from the starting day with respect to tagging and duration from this day. The figure represents the AIC values of the binomial GLMs explaining pod status change from tagging for each period of influence. On the starting date axis, zero corresponds to the tagging date of pods of 3 to 10 cm in length. Circles indicate the lowest AIC value and the best microclimatic predictors of pod status change (period of influence). The presence of a delimited surrounded black to gray zone indicates a zone of decreasing influence of the variable. Gray scale on the right represents the AIC values. Absence of circle indicates that no clear influence zone was identified.*

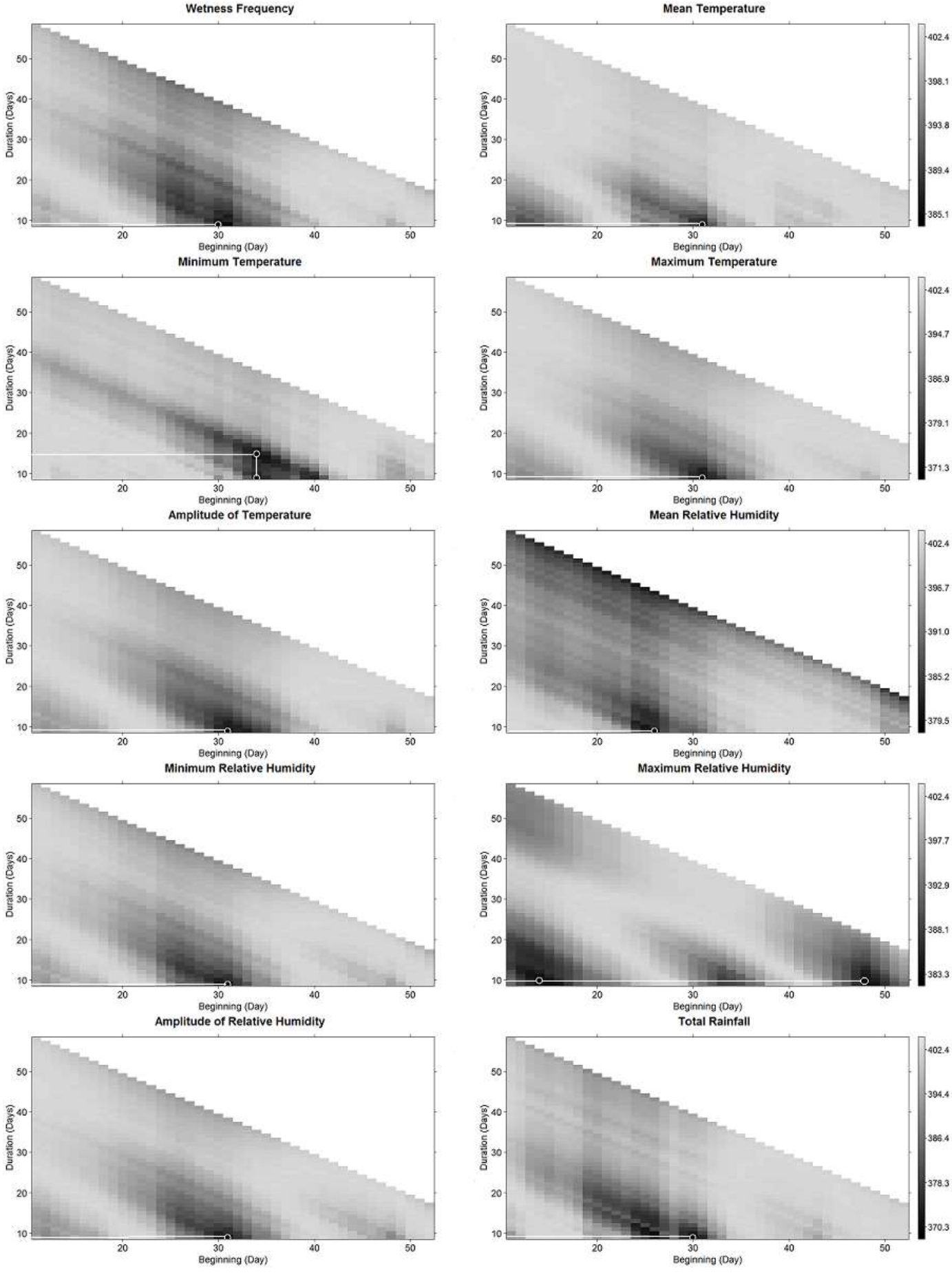

Wetness Frequency
Mean Temperature
Minimum Temperature
Maximum Temperature
Amplitude of Temperature
Mean Relative Humidity
Minimum Relative Humidity
Maximum Relative Humidity
Amplitude of Relative Humidity
Total Rainfall
Duration (Days)
Beginning (Day)
10
20
30
40
50
402.4
398.1
393.8
389.4
385.1
394.7
386.9
379.1
371.3
396.7
391.0
385.2
379.5
397.7
392.9
388.1
383.3
394.4
386.4
378.3
370.3

*Figure* 4. *Period of influence of each daily variable on pod status change, from diseased with no signs of sporulation to diseased with sporulated lesions, 60 to 70 days after tagging, for CC-137. By period of influence, we meant from the starting day with respect to tagging and duration from this day. The figure represents the AIC values of the binomial GLM, explaining pod status change from tagging for each period of influence. On the starting date axis, zero corresponds to the tagging date of pods 3 to 10 cm in length. Circles indicate the lowest AIC value and the best microclimatic predictors of pod status change (period of influence). The presence of a delimited surrounded black to gray zone indicates a zone of decreasing influence of the variable. Gray scale on the right represents the AIC values. Absence of circle indicates that no clear influence zone was identified.*

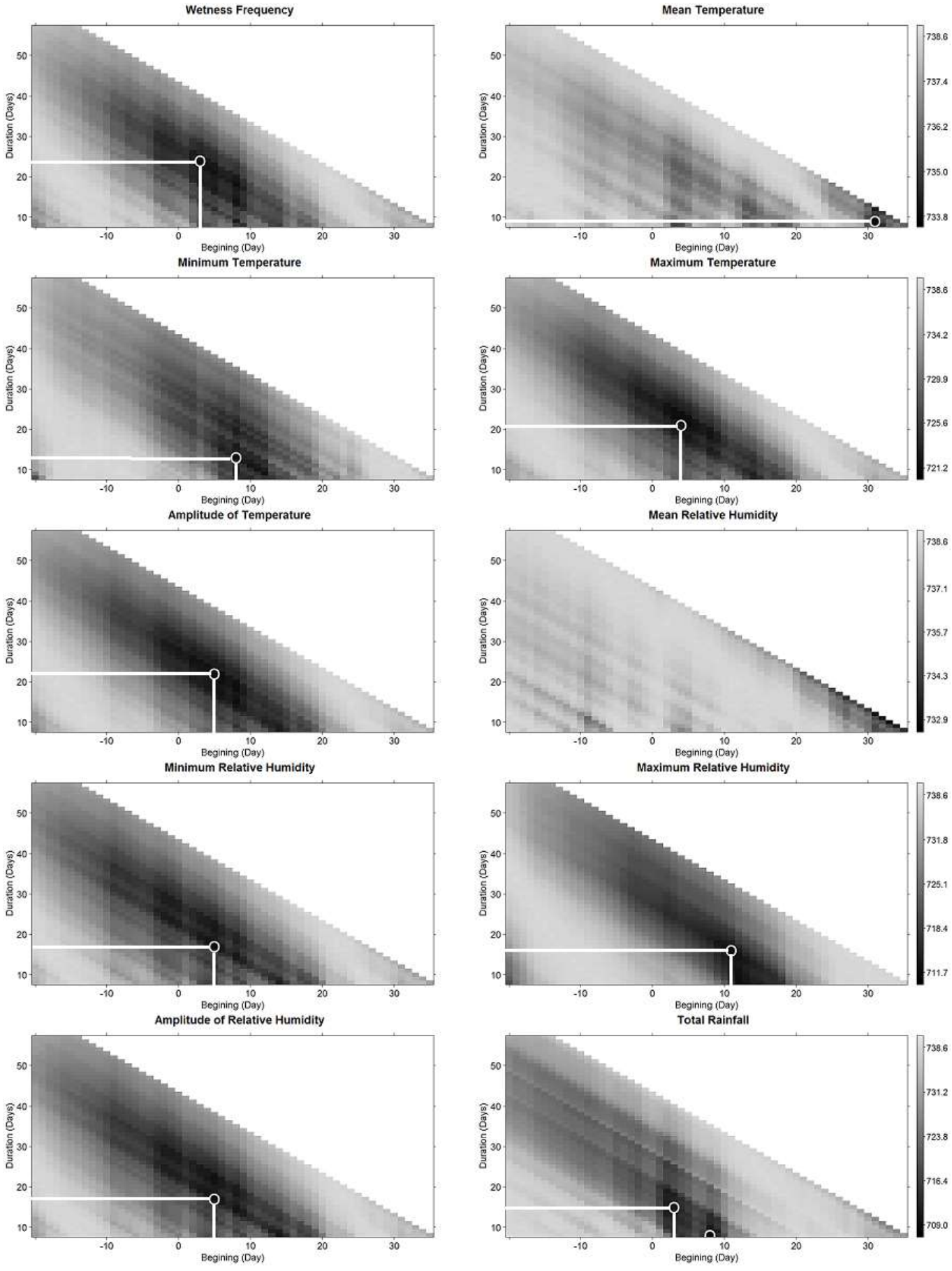
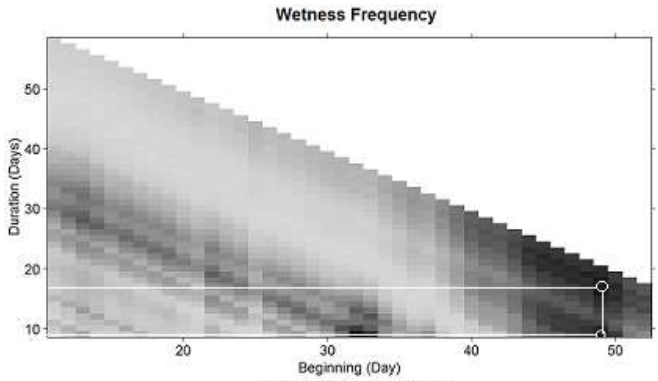
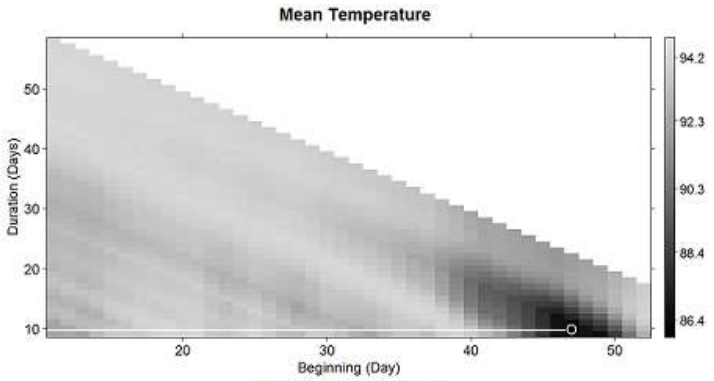
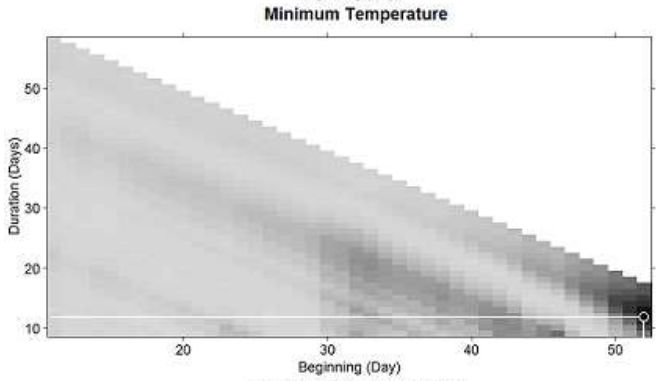
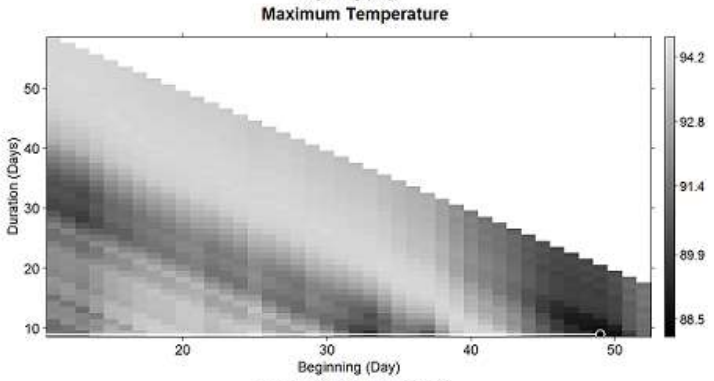

Wetness Frequency
Mean Temperature
Minimum Temperature
Maximum Temperature
Amplitude of Temperature
Mean Relative Humidity
Minimum Relative Humidity
Maximum Relative Humidity
Amplitude of Relative Humidity
Total Rainfall
Duration (Days)
Begining (Day)
10
20
30
40
50
-10
0
10
20
30
738.6
737.4
736.2
735.0
733.8
738.6
734.2
729.9
725.6
721.2
738.6
737.1
735.7
734.3
732.9
738.6
731.8
725.1
718.4
711.7
738.6
731.2
723.8
716.4
709.0

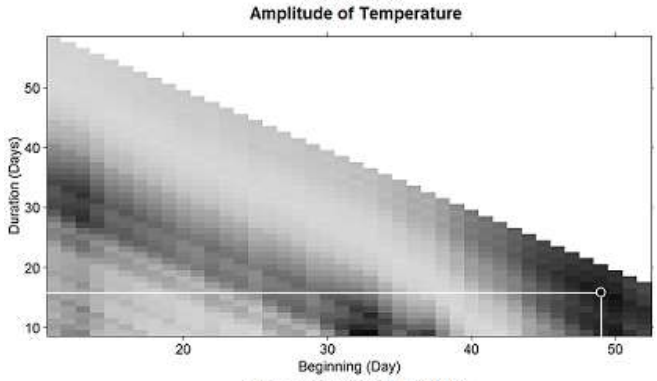
*Figure* 5. *Period of influence of each daily variable on pod status change, from healthy to diseased with no signs of sporulation, 40 to 50 days after tagging, for CATIE-R4. By period of influence, we meant from the starting day with respect to tagging and duration from this day. The figure represents the AIC values of the binomial GLMs explaining pod status change from tagging for each period of influence. On the starting date axis, zero corresponds to the tagging date of pods 3 to 10 cm in length. Circles indicate the lowest AIC value and the best microclimatic predictors of pod status change (period of influence). The presence of a delimited surrounded black to gray zone indicates a zone of decreasing influence of the variable. Gray scale on the right represents the AIC values. Absence of circle indicates that no clear influence zone was identified.*

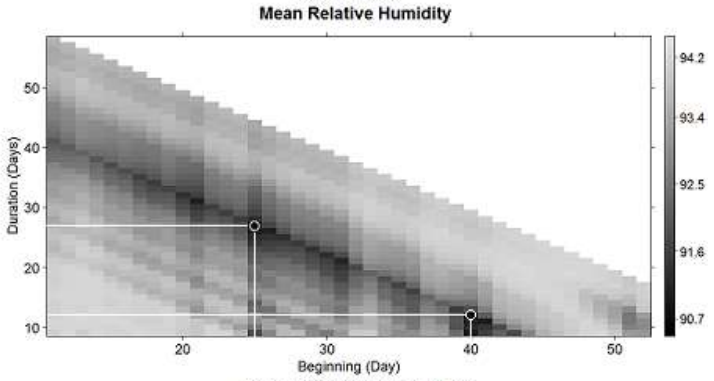

Wetness Frequency
Duration (Days)
Beginning (Day)

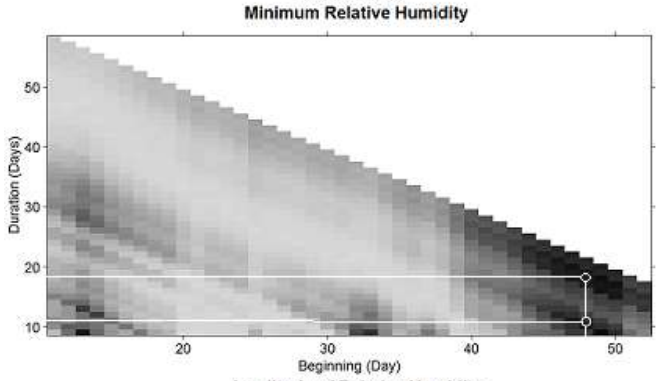

Mean Temperature
Duration (Days)
Beginning (Day)
94.2
92.3
90.3
88.4
86.4

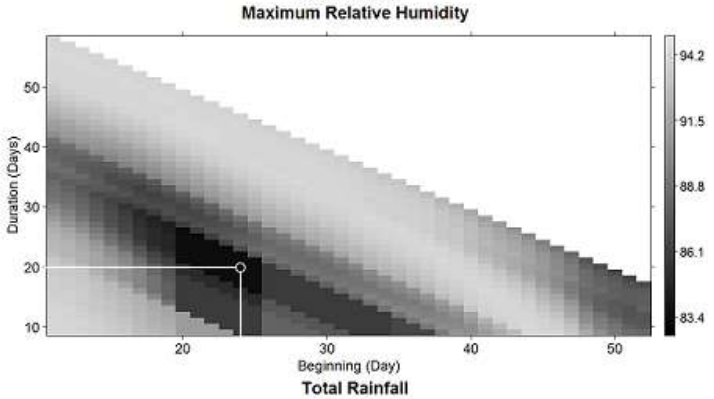

Minimum Temperature
Duration (Days)
Beginning (Day)

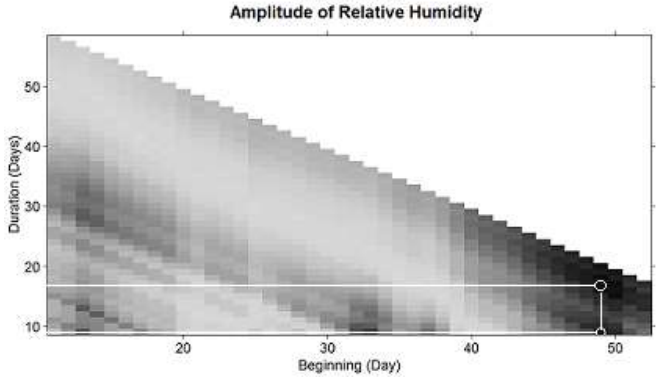

Maximum Temperature
Duration (Days)
Beginning (Day)
94.2
92.8
91.4
89.9
88.5


Amplitude of Temperature
Duration (Days)
Beginning (Day)


Mean Relative Humidity
Duration (Days)
Beginning (Day)
94.2
93.4
92.5
91.6
90.7


Minimum Relative Humidity
Duration (Days)
Beginning (Day)


Maximum Relative Humidity
Duration (Days)
Beginning (Day)
94.2
91.5
88.8
86.1
83.4


Amplitude of Relative Humidity
Duration (Days)
Beginning (Day)

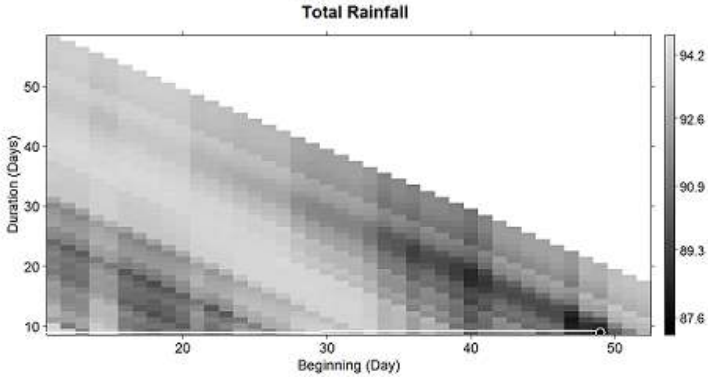

Total Rainfall
Duration (Days)
Beginning (Day)
94.2
92.6
90.9
89.3
87.6

*Figure* 6. *Period of influence of each daily variable on pod status change, from diseased with no signs of sporulation to diseased with sporulated lesions, 60 to 70 days after tagging, for CATIE-R4. By period of influence, we meant from the starting day with respect to tagging and duration from this day. The figure represents the AIC values of the binomial GLMs explaining pod status change from tagging for each period of influence. On the starting date axis, zero corresponds to the tagging date of pods 3 to 10 cm in length. Circles indicate the lowest AIC value and the best microclimatic predictors of pod status change (period of influence). The presence of a delimited surrounded black to gray zone indicates a zone of decreasing influence of the variable. Gray scale on the right represents the AIC values. Absence of circle indicates that no clear influence zone was identified.*

## RESULTS

### Resistance clonal behavior during key moments

Figure 7 represents four different pod generations (6, 19, 24 and 37), in which some of these clones showed an uncommon resistance behavior. During Generation 6, as shown in Figure 7a, the three clones are at low incidence, especially Pound-7, which reduced its incidence by almost half. During Generation 19 (Figure 7b, the contrary occurred, and all of the clones showed high incidences, especially CATIE-R4, which reached almost 60% of incidence—completely unexpected. For Generations 24 and 37 (Figures 7c and 7d), it was observed that CC-137 incidence could fluctuate even when the other two clones presented expected behavior.

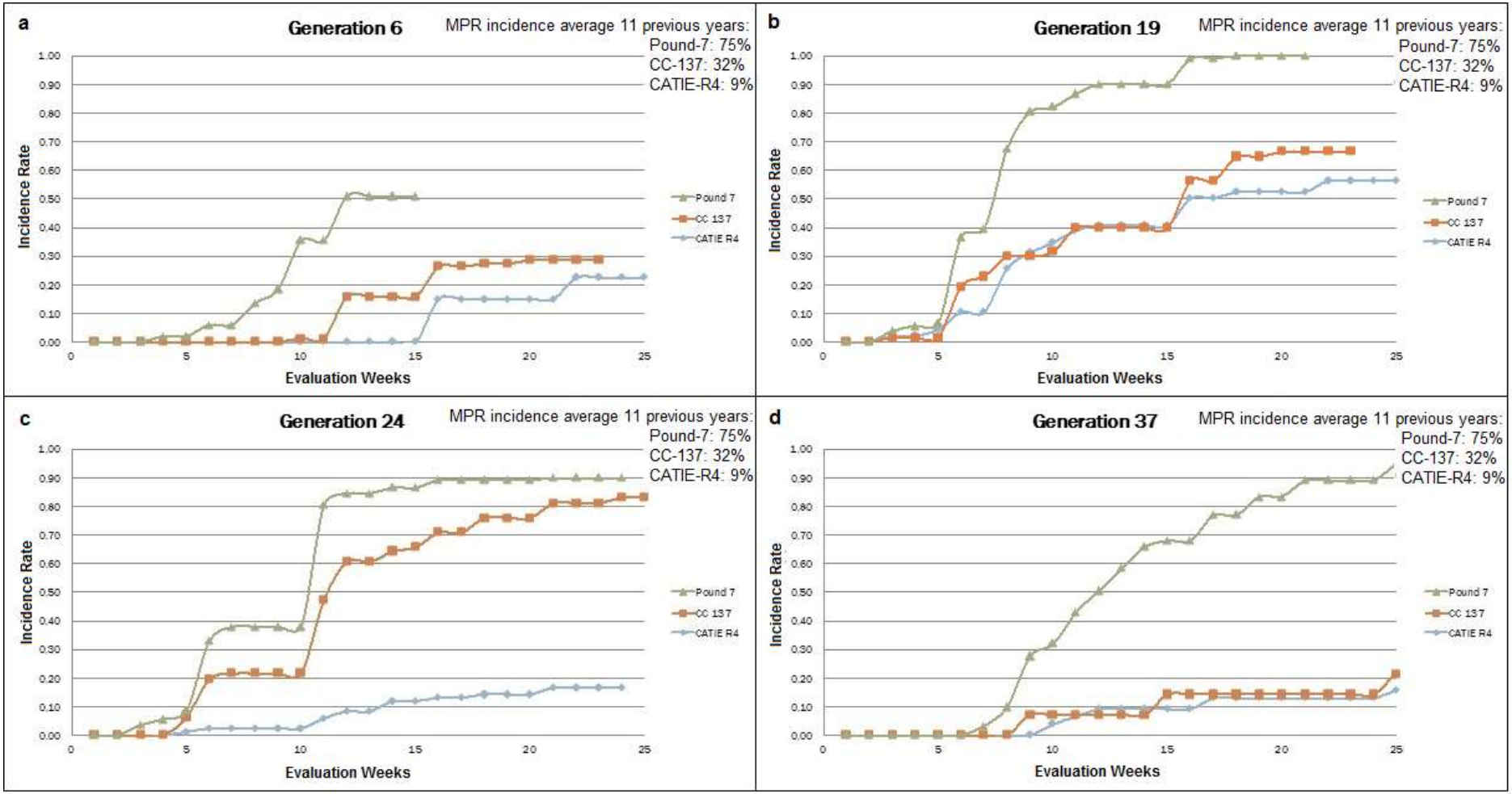



*Figure 7. Reduction of the incidence of the clones Pound-7, CC-137 and CATIE-R4 compared with their averages: a. Generation 6 (young pods 3 to 10cm in length tagged July 8–14, 2012), b. Generation 19 (young pods 3 to 10 cm in length tagged October 7–13, 2012), c. Generation 24 (young pods 3 to 10cm in length tagged November 11–17, 2012), d. Generation 37 (young pods 3 to 10cm in length tagged February 10–16, 2013).*

**Selection of the period of effect of each microclimatic variable**

In the case of H→D change of clone CC-137, the period of influence of almost all of the variables included the period from -20 to -2 d.a.t., except for minimum temperature (from 12 to 26 d.a.t., Table 1) and total rainfall (from 8 to 18 d.a.t., Table 1). In the case of D→S change, the period of influence of all variables included the period from 28 to 58 d.a.t. (Table 2).

*Table 1. Selected microclimatic predictors (starting date and duration) of pod status change from healthy to diseased with no sign of sporulation, from 40 to 50 days after tagging, for clone CC-137.*

| | | Tagging | | |
|---|---|---|---|---|
| | | Days *before* tagging | Days *after* tagging | Studied period |
| ID | Variables | -20 -18 -16 -14 -12 -10 -8 -6 -4 -2 | 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 | 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 |
| WF -15 to -3 | Wetness frequency | -16 -14 -12 -10 -8 -6 -4 -2 | | |
| Tmean -15 to -2 | Mean temperature | -16 -14 -12 -10 -8 -6 -4 -2 | | |
| Tmin 12 to 21 | Minimum temperature | | 12 14 16 18 20 22 | |
| Tmin 12 to 26 | Minimum temperature | | 12 14 16 18 20 22 24 26 | |
| Tmax -15 to -6 | Maximum temperature | -16 -14 -12 -10 -8 -6 | | |
| Tmax -15 to -1 | Maximum temperature | -16 -14 -12 -10 -8 -6 -4 -2 | | |
| Tamp -15 to -2 | Amplitude of temperature | -16 -14 -12 -10 -8 -6 -4 -2 | | |
| RHmean -15to -6 | Mean Relative Humidity | -16 -14 -12 -10 -8 -6 | | |
| RHmin -15 to -6 | Minimum Relative Humidity | -16 -14 -12 -10 -8 -6 | | |
| RHmax -20 to 7 | Maximum Relative Humidity | -20 -18 -16 -14 -12 -10 -8 -6 | | |
| RHamp -15 to -6 | Amplitude Relative Humidity | -16 -14 -12 -10 -8 -6 | | |
| TR -16 to -8 | Total Rainfall | -16 -14 -12 -10 -8 | | |
| TR 8 to 18 | Total Rainfall | | 8 10 12 14 16 18 | |

*Table 2. Selected microclimatic predictors (starting date and duration) of pod status change from diseased with no sign of sporulation to diseased with sporulated lesions, from 60 to 70 days after tagging, for clone CC-137*

| | | Tagging | | |
|---|---|---|---|---|
| | | Days *before* tagging | Days *after* tagging | Studied period |
| ID | Variables | -20 -18 -16 -14 -12 -10 -8 -6 -4 -2 | 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 | 60 62 64 66 68 70 |
| WF 30 to 49 | Wetness frequency | | 30 32 34 36 38 40 42 44 46 48 | |
| Tmean 48 to 59 | Mean temperature | | 48 50 52 54 56 58 | |
| Tmin 35 to 58 | Minimum temperature | | 36 38 40 42 44 46 48 50 52 54 56 58 | |
| Tmin 46 to 59 | Minimum temperature | | 46 48 50 52 54 56 58 | |
| Tmax 37 to 46 | Maximum temperature | | 38 40 42 44 46 | |
| Tamp 37 to 47 | Amplitude of temperatura | | 38 40 42 44 46 48 | |
| RHmin 38 to 49 | Minimum Relative Humidity | | 38 40 42 44 46 48 | |
| TR 28 to 40 | Total Rainfall | | 28 30 32 34 36 38 40 | |

In the case of H→D change of clone CATIE-R4, the period of influence, of almost all variables included the period from 2 to 28 d.a.t., except for mean temperature (from 30 to 40 d.a.t., Table 3). In the case of D→S change, the period of influence of almost all variables included the period from 46 to 68 d.a.t., except for mean, minimum and maximum relative humidity that included the period from 24 to 52 d.a.t. (Table 4).

*Table 3. Selected microclimatic predictors (starting date and duration) of pod status change from healthy to diseased with no sign of sporulation, from 40 to 50 days after tagging, for clone CATIE-R4.*

| | | Tagging | | |
|---|---|---|---|---|
| | | Days *before* tagging | Days *after* tagging | Studied period |
| ID | Variables | -20 -18 -16 -14 -12 -10 -8 -6 -4 -2 | 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 | 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 |
| $WF_{3\ to\ 27}$ | Wetness frequency | | 2 4 6 8 10 12 14 16 18 20 22 24 26 28 | |
| $Tmean_{31\ to\ 40}$ | Mean temperature | | 30 32 34 36 38 | 40 |
| $Tmin_{8\ to\ 21}$ | Minimum temperature | | 8 10 12 14 16 18 20 22 | |
| $Tmax_{4\ to\ 25}$ | Maximum temperature | | 4 6 8 10 12 14 16 18 20 22 24 26 | |
| $Tamp_{5\ to\ 27}$ | Amplitude of temperature | | 4 6 8 10 12 14 16 18 20 22 24 26 28 | |
| $RHmin_{5\ to\ 22}$ | Minimum Relative Humidity | | 4 6 8 10 12 14 16 18 20 22 | |
| $RHmax_{11\ to\ 27}$ | Maximum Relative Humidity | | 12 14 16 18 20 22 24 26 28 | |
| $RHamp_{5\ to\ 22}$ | Amplitude Relative Humidity | | 4 6 8 10 12 14 16 18 20 22 | |
| $TR_{8\ to\ 16}$ | Total Rainfall | | 8 10 12 14 16 | |
| $TR_{3\ to\ 18}$ | Total Rainfall | | 2 4 6 8 10 12 14 16 18 | |

*Table 4. Selected microclimatic predictors (starting date and duration) of pod status change from diseased with no sign of sporulation to diseased with sporulated lesions, from 60 to 70 days after tagging, for clone CATIE-R4.*

| | | Tagging | | |
|---|---|---|---|---|
| | | Days *before* tagging | Days *after* tagging | Studied period |
| ID | Variables | -20 -18 -16 -14 -12 -10 -8 -6 -4 -2 | 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 | 60 62 64 66 68 70 |
| $WF_{49\ to\ 58}$ | Wetness frequency | | 48 50 52 54 56 58 | |
| $WF_{49\ to\ 58}$ | Wetness frequency | | 48 50 52 54 56 58 | 60 62 64 66 |
| $Tmean_{47\ to\ 57}$ | Mean temperature | | 46 48 50 52 54 56 58 | |
| $Tmin_{52\ to\ 64}$ | Minimum temperature | | 52 54 56 58 | 60 62 64 |
| $Tmax_{49\ to\ 58}$ | Maximum temperature | | 48 50 52 54 56 58 | |
| $Tamp_{49\ to\ 65}$ | Amplitude of temperature | | 48 50 52 54 56 58 | 60 62 64 66 |
| $RHmean_{25\ to\ 52}$ | Mean Relative Humidity | | 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 | |
| $RHmean_{40\ to\ 52}$ | Mean Relative Humidity | | 40 42 44 46 48 50 52 | |
| $RHmin_{48\ to\ 59}$ | Minimum Relative Humidity | | 48 50 52 54 56 58 | 60 |
| $RHmin_{48\ to\ 68}$ | Minimum Relative Humidity | | 48 50 52 54 56 58 | 60 62 64 66 68 |
| $RHmax_{24\ to\ 44}$ | Maximum Relative Humidity | | 24 26 28 30 32 34 36 38 40 42 44 | |
| $RHapm_{49\ to\ 58}$ | Amplitude of Relative Humidity | | 48 50 52 54 56 58 | |
| $RHapm_{49\ to\ 67}$ | Amplitude of Relative Humidity | | 48 50 52 54 56 58 | 60 62 64 66 68 |
| $TR_{49\ to\ 58}$ | Total Rainfall | | 48 50 52 54 56 58 | |

**Best fitted models construction**

After model selection, for clone CC-137, $WF_{-15\ to\ -3}$, $Tmin_{12\ to\ 26}$, and $RHmin_{-15\ to\ -6}$ and the square of the three of them significantly predicted H→D change. $Tmin_{34\ to\ 49}$, its square value and $TR_{30\ to\ 39}$ significantly predicted D→S change. Model predictions of H→D change (Figure 8a and 8b) show that $WF_{-15\ to\ -3}$ and $RHmin_{-15\ to\ -6}$ had a positive relationship with the status change probability. On the other hand, $Tmin_{12\ to\ 26}$ had a negative relationship with status change probability. As with the clone Pound 7, the probability of change was low, with a maximum value of 0.55 predicted by the model. Model predictions for D→S change (Figure 8c) show that $Tmin_{34\ to\ 49}$ and $TR_{30\ to\ 39}$ were the most explanatory variables (microclimatic predictors) for explaining the probability of status change. Both variables had a negative relationship with the status change probability. In La Lola weather conditions, the highest change probability (0.6) was found when minimum temperature was about 20°C (34 to 49 d.a.t.) and total rainfall was 0 (30 to 39 d.a.t.).

For clone CATIE-R4, $Tmin_{8\ to\ 21}$ and its square value and $Tmax_{4\ to\ 25}$ significantly predicted H→D change. $Tmax_{49\ to\ 58}$ and $RHmean_{40\ to\ 52}$ and the square value of both of them significantly predicted D→S change. Model predictions of H→D change (Figure 9a) show that $Tmin_{8\ to\ 21}$ had a negative relationship with the status change probability. On the other hand, $Tmax_{4\ to\ 25}$ had a positive relationship with the status change probability. For this clone, the probability of change was considerably low when compared with the other two clones, with a maximum value of 0.14 predicted by the model. Model predictions for D→S change (Figure 9b) showed that $Tmax_{49\ to\ 58}$ and $RHmean_{40\ to\ 52}$ were the most explanatory variables (microclimatic

predictors) for explaining the probability of status change. $Tmax_{49\ to\ 58}$ had a negative relationship with the status change probability and $RHmean_{40\ to\ 52}$ had a positive relationship. In La Lola weather conditions, the highest change probability (0.5) was found when maximum temperature was about 23°C (49 to 58 d.a.t.) and mean relative humidity was 97% (40 to 52 d.a.t.).

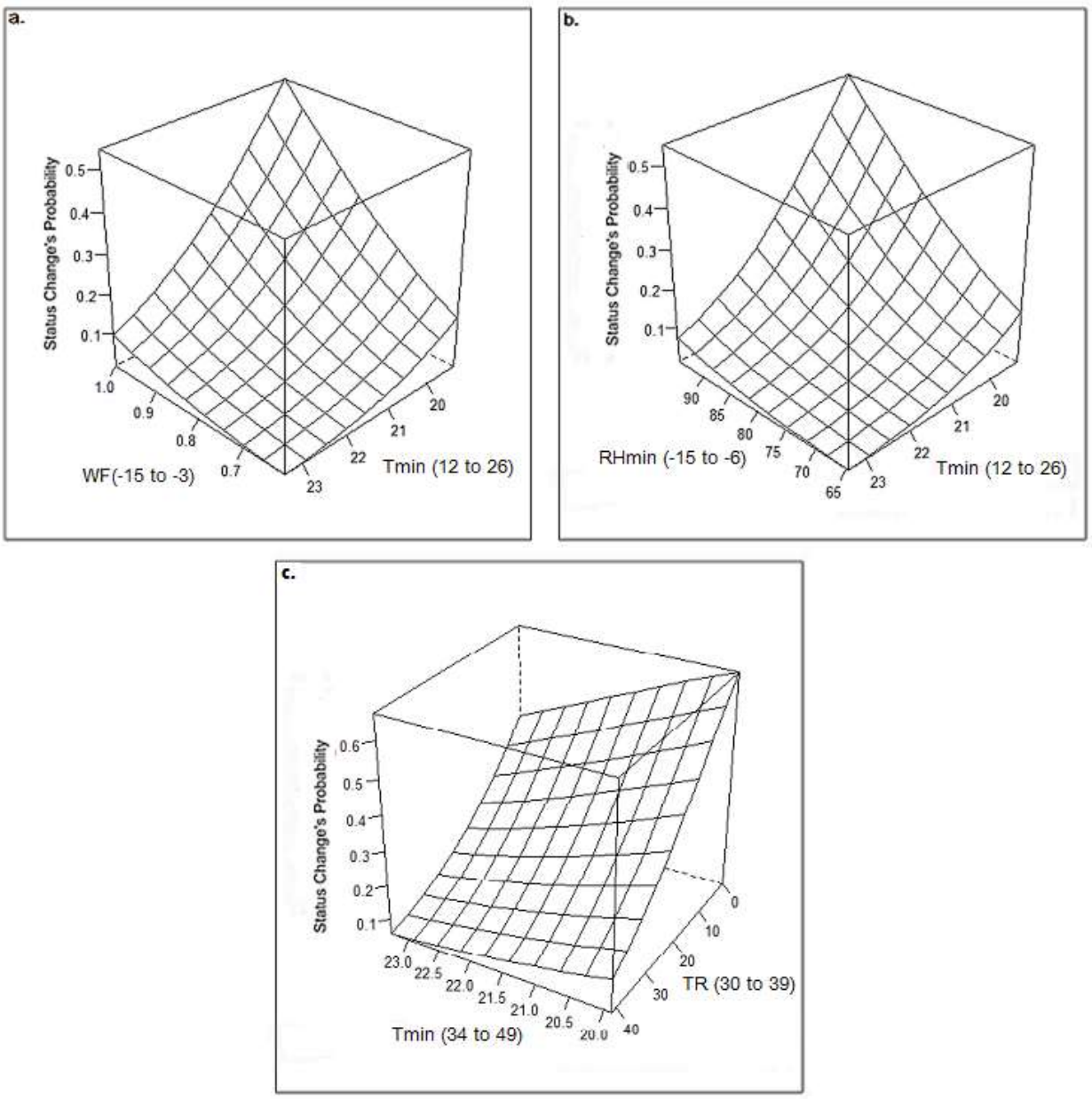



*Figure* 8. ***Best fitted model predicts for CC-137:*** *a. status change probability from healthy to diseased pod without sporulation between 40 to 50 days after tagging, b. status change probability from healthy to diseased pod without sporulation between 40 to 50 days after tagging, c. status change probability from diseased pod without sporulation to diseased pod with sporulated lesions between 60 to 70 days after tagging. Numbers between parentheses indicate the range of days of influence of each variable with respect to tagging.*

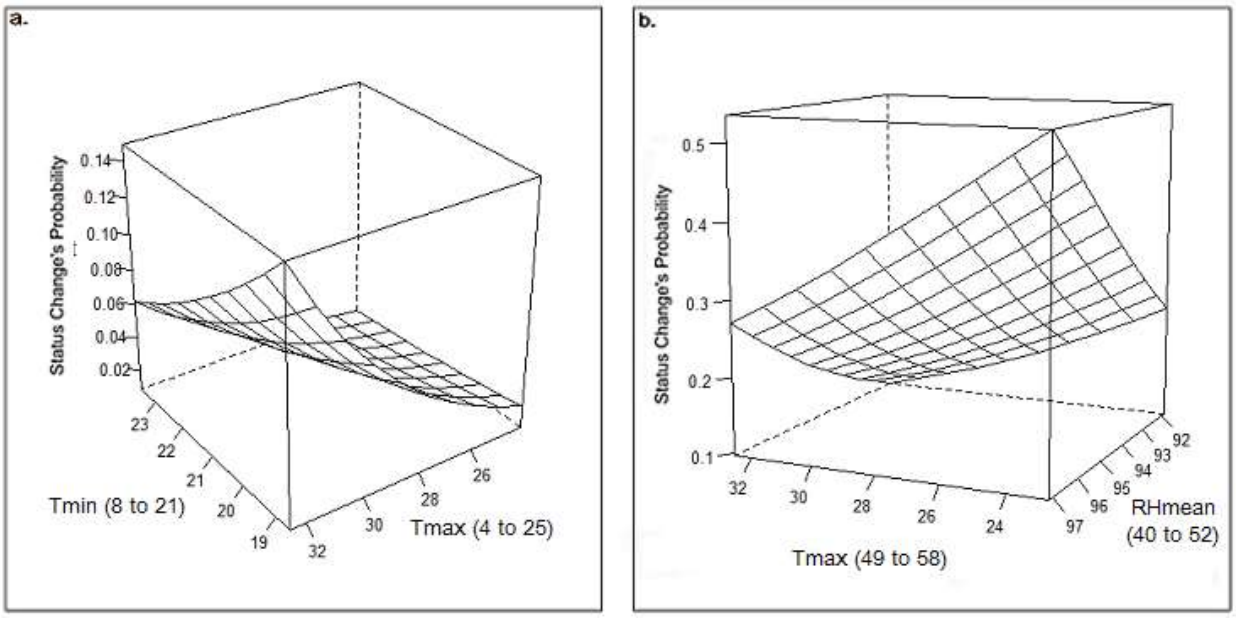



*Figure* 9. ***Best fitted model predicts for CATIE-R4:*** *a. status change probability from healthy to diseased pod without sporulation between 40 to 50 days after tagging, b status change probability from diseased pod without sporulation to diseased pod with sporulated lesions between 60 to 70 days after tagging. Numbers between parentheses indicate the range of days of influence of each variable with respect to tagging.*

## DISCUSSION

### Genotype-environment interaction

Figure 7 clearly illustrates the existence of an interaction between cacao incomplete resistance to MPR and the environment, showing uncommon behaviors of the three genotypes against the pathogen in determinate generations. Differences within generations are due to the environment, especially climate. Figures 7a and 7b, corresponding to Generations 6 and 19, respectively, show that the three clones could significantly increase or decrease their MPR incidence as a group due to environmental conditions. Differences in resistance among these clones lie in the number of resistant genes accumulated; however, the resistance of the three may be affected under certain environmental condition. This influence is reported as typical for this type of resistance (Zadoks and Van Leur 1983) and has been described in other pathosystems by several authors (Eskes 1982; Eskes and Toma-Braghini 1982; Bonman 1992; Banito *et al.* 2008; Rubiales *et al.* 2012). On the other hand, Figures 7c and 7d, corresponding to Generations 24 and 37, respectively, show a different scenario. Only CC-137, considered a moderately resistant clone, presents an atypical behavior. This result confirms the conclusion made by Porras Umaña (1985) and confirmed by Phillips (1986) that highly resistant or highly susceptible genotypes are very stable, while intermediate clones, such as CC-137, vary according to climatic conditions and inoculum pressure. Our result also agrees with those obtained by Price *et al.* (2004) describing the resistance of *Euthamia graminifolia* against leaf rust.

### Resistance mechanisms against MPR

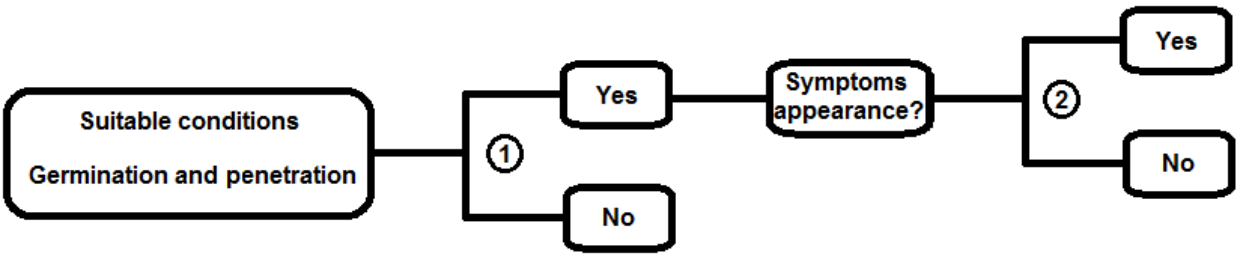


*Figure* 10. *MPR infection diagram. 1 and 2 indicate the moment of infection and symptoms onset.*

The analyses of the influence of the microclimatic variables highlighted two important events where resistance strategies could be developed. These events are indicated by numbers 1 and 2 in Figure 10. Number 1 indicates the infection (fungal germination and penetration), where PAMP-triggered immunity (PTI) could be activated. Number 2 indicates the symptoms onset, where the effector-triggered immunity (ETI) could occur. According to our models (Figures 8a, 8b and 8c), success of these two events responds to the effect of humidity (wetness and relative humidity) and temperature, respectively. However, according to Figure 9a, not any water-related variables showed up for clone CATIE-R4, suggesting that germination and penetration are not critical events for this clone. We considered that this is not what is really happening, that the real reason why this influence did not show up is because of the low numbers of CATIE-R4 pods that showed symptoms. Penetration success was not determined in this study, so the only proof we had was the appearance of the symptoms, and thus this influence was left out for CATIE-R4. For this reason, discarding humidity influence over the infection moment for this clone is not correct. Nonetheless, we considered that none of the clones present PTI as a defense mechanism against spore germination and penetration, i.e. *M. roreri* can easily penetrate the pods. Host resistance mechanisms resulting from the ETI are triggered internally and against colonization, where temperatures influence the success of these strategies. For CATIE-R4, large amplitudes of temperatures favor fungal colonization, evidenced by the appearance of symptoms, although status change probabilities were very low.

For CC-137, water conditions within the first month influenced the moment of infection (germination and penetration). After penetration, low minimum temperatures (about 20°C) elevated the probability of the pods to show symptoms. We considered that this variable influenced the ETI against fugal colonization. However, this resistance strategy was not as effective as the one developed by CATIE-R4, and this could suggest that CC-137 accumulates a minor number of resistance genes, so its ETI against the pathogen is less effective. In addition, the different response of this clone according to the influence of environmental conditions shown in Figure 7 suggests that its resistance genes are different from CATIE-R4 and Pound-7 resistance genes.

**Resistant clones' stability: the case of CATIE-R4**

After field observations and the result of our analyses, CATIE-R4 was found to be a very stable, highly resistant clone. This clone is considered a promising material; however, its resistance stability should be proved in special environments where the large temperature ranges that favor fungal colonization are the norm. The CATIE-R4 resistance strategy consists of the interruption of fungal colonization as an ETI strategy. This interruption also avoids fungal reproduction since we considered that *M. roreri* has difficulties sporulating over CATIE-R4 pods, causing inoculum suppression. For CC-137 and Pound-7, common sporulation was observed normally in the field, with mycelium and spores as commonly reported in the literature (Thévenin and Trocmé 1996). This was not the case of CATIE-R4. Even when most of the CATIE-R4 diseased pods sporulated, this sporulation was different from that in any other susceptible clone. Mycelium and spores were lighter in terms of color and thickness. Sporulation observed could correspond to other secondary fungi. In this way, effect differences of the variables within clones could be explained. For CC-137, temperatures and rainfall presented normal effects, i.e. status change probability was higher when minimum temperatures and rainfall decreased and maximum temperature increased. In this way, as mentioned in the part I discussion, these conditions were favorable for dissemination. The opposite happened with CATIE-R4, where low maximum temperature and high mean relative humidity elevated the probability of status change. This could be secondary/decomposer fungi that have different environmental requirements, favored by an increment of relative humidity and lower temperatures (Lodge and Cantrell 1995). The CATIE-R4 infection process should be carefully studied to determine what happened in the interior of the pods and whether the apparent sporulation corresponds to *M. roreri.*

**REFERENCES**


Avelino, J; Cabut, S; Barboza, B; Barquero, M; Alfaro, R; Esquivel, C; Durand, J-F; Cilas, C. 2007. Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica Phytopathology 97(12):1532-1542.

Banito, A; Kpemoua, K; Wydra, K. 2008. Expression of resistance and tolerance of cassava genotypes to bacterial blight determined by genotype x environment interactions Journal of Plant Diseases and Protection 115(4):152-161.

Bonman, J. 1992. Durable resistance to rice blast disease—environmental influences. Springer. 115-123 p.

Eskes, A. 1982. The effect of light intensity on incomplete resistance of coffee to Hemileia vastatrix Netherlands Journal of Plant Pathology 88(5):191-202.

Eskes, AB; Toma-Braghini, M. 1982. The effect of leaf age on incomplete resistance of coffee to Hemileia vastatrix Netherlands Journal of Plant Pathology 88(6):219-230.

Jones, JD; Dangl, JL. 2006. The plant immune system Nature 444(7117):323-329.

Leandro-Muñoz, ME; Tixier, P; Germon, A; Rakotobe, V; Phillips-Mora, W; Maximova, S; Avelino, J. 2017. Effects of microclimatic variables on the symptoms and signs onset of Moniliophthora roreri, causal agent of Moniliophthora pod rot in cacao PLoS One 12(10):e0184638. Disponible en https://doi.org/10.1371/journal.pone.0184638 doi 10.1371/journal.pone.0184638

Lodge, DJ; Cantrell, S. 1995. Fungal communities in wet tropical forests: variation in time and space Canadian Journal of Botany 73(S1):1391-1398.

Parlevliet, J. 1979. Components of resistance that reduce the rate of epidemic development Annual Review of Phytopathology 17(1):203-222.

Phillips-Mora, W; Arciniegas-Leal, A; Mata-Quirós, A; Motamayor-Arias, J. 2013. Catalogue of cacao clones selected by CATIE for commercial plantings. 1st ed. Turrialba, CR, Tropical Agricultural Research and Higher Education Center (CATIE). 68 p. (Technical series. Technical manual).

Phillips, W. 1986. Evaluación de la resistencia de cultivares de cacao (*Theobroma cacao* L.) a *Moniliophthora roreri* (Cif. y Par.) Evans et al. Tesis Turrialba, Costa Rica. , Universidad de Costa Rica / Centro Agronómico Tropical de Investigación y Enseñanza. (Costa Rica). Disponible en http://orton.catie.ac.cr/repdoc/A1720e/A1720e.pdf

Porras Umaña, V. 1985. Determinacion de la estabilidad de la resistencia a *Monilia roreri* en cultivares de cacao en dos zonas de Costa Rica. Tesis Turrialba, Costa Rica), Universidad de Costa Rica, San José. Centro Agronómico Tropical de Investigación y Enseñanza.

Price, J; Bever, J; Clay, K. 2004. Genotype, environment, and genotype by environment interactions determine quantitative resistance to leaf rust (Coleosporium asterum) in Euthamia graminifolia (Asteraceae) New Phytologist 162:729-743.

Rubiales, D; Ávila, CM; Sillero, JC; Hybl, M; Narits, L; Sass, O; Flores, F. 2012. Identification and multi-environment validation of resistance to Ascochyta fabae in faba bean (Vicia faba) Field Crops Research 126:165-170.

Thévenin, JM; Trocmé, O. 1996. La moniliose du cacaoyer. La moniliasis del cacao. 397-406 p. (Plantations, recherche, développement).

Waller, JM; Lenné, JM; Waller, SJ. 2002. Plant pathologist's pocketbook. CABI.

Wang, A; Avelino, J. 1999. El Ojo de Gallo del Cafeto (*Mycena citricolor*):

Zadoks, J; Van Leur, J. 1983. Durable resistance and host-pathogen-environment interaction. Springer. 125-140 p.